

AMENDED CLAIMS

1. (twice amended) A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein at least one of the one or more nutrient media comprises one or more enhancement agents selected from the group consisting of (a) jasmonate-related compounds (an) alkyl esters thereof, (b) antiethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

2. The method of claim 1, wherein the one or more nutrient media contain an antiethylene agent which is a silver-containing compound, or a silver complex, or a silver ion.

3. (twice amended) The method of claim 1, wherein a jasmonate-related compound or an alkyl ester thereof is added to the one or more nutrient media.

6. (amended) The method of claim 3, wherein the jasmonate-related compound is in a concentration from 10^{-5} to 2×10^{-4} M.

7. (amended) The method of claim 3, wherein the jasmonate-related compound is at least one compound selected from the group consisting of jasmonic acid, ^{and} dihydrojasmonic acid.

8. The method of claim 3, wherein the jasmonate-related compound is at least one compound selected from the group consisting of jasmonic acid ^{and} alkyl esters of jasmonic acid.

9. (twice amended) The method of claim 8, wherein said alkyl ester of jasmonic acid comprises an alkyl group esterified to jasmonic acid wherein said alkyl group has from one to four carbon atoms.

10. The method of claim 8, wherein the alkyl group esterified to jasmonic acid has one carbon atom.

11. The method of claim 3, wherein the cells are cultured in the presence of heavy metal ions, heavy metal complexes, or heavy metal-containing compounds.

12. (amended) The method of claim 11, wherein the heavy metal ions are cobalt ions, the heavy metal complexes are cobalt complexes, and the heavy metal-containing compounds are cobalt-containing compounds.

13. (amended) The method of claim 3, wherein the cells are cultured in the presence of an antiethylene agent.

14. The method of claim 13, wherein the antiethylene agent is an ethylene-biosynthesis antagonist.

15. The method of claim 14, wherein the ethylene-biosynthesis antagonist is a compound which inhibits ACC synthase, ACC oxidase, or ethylene oxidase.

16. The method of claim 14, wherein the ethylene-biosynthesis antagonist is acetylsalicylic acid or aminooxyacetic acid.

17. The method of claim 13, wherein the antiethylene agent is an ethylene-action antagonist.

18. The method of claim 17, wherein the ethylene-action antagonist is a silver-containing compound, a silver complex or silver ion.

19. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver thiosulfate, silver chloride, and silver oxide.

20. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver phosphate, silver benzoate, toluenesulfonic acid silver salt, silver acetate, silver nitrate, and silver sulfate.

21. (amended) The method of claim 18, wherein the silver-containing compound is at least one compound selected from the group consisting of silver pentafluoropropionate, silver cyanate, lactic acid silver salt, silver hexafluorophosphate, citric acid trisilver salt, and silver nitrite.

24. (amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, and silver-containing compounds is 10 μ M – 100 μ M

25. (amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, and silver-containing compounds is 50 μ M.

26. (amended) The method of claim 18, wherein the concentration of silver ions, silver complexes, and silver-containing compounds is 10 μ M.

27. The method of claim 18, wherein the molar ratio of silver to jasmonic acid in the one or more nutrient media is less than 9.5.

28. The method of claim 1, wherein the one or more nutrient media contain an inhibitor of phenylpropanoid metabolism.

29. The method of claim 28, wherein the inhibitor of phenylpropanoid metabolism is selected from the group consisting of 3,4-methylenedioxynitrocinnamic acid, 3,4-methylenedioxycinnamic acid, 3,4-methylenedioxy-phenylpropionic acid, 3,4-methylenedioxyphenylacetic acid, 3,4-methylenedioxybenzoic acid, 3,4,-trans-dimethoxycinnamic acid, 4-hydroxycinnamic acid, phenylpropionic acid, fluorophenylalanine, 1-aminobenzotriazole, 2-hydroxy-4,6-dimethoxybenzoic acid, SKF-525A, ammonium oxalate, vinylimidazole, diethyldithiocarbamic acid, and sinapic acid.

30. The method of claim 1, wherein the one or more nutrient media contain at least one enhancement agent selected from each of at least two of the following classes of enhancement agents: (a) jasmonic acid or an alkyl ester thereof, (b) antiethylene agents, and (c) inhibitors of phenylpropanoid metabolism.

31. The method of claim 30, wherein the jasmonic acid alkyl ester is methyl jasmonate.

32. The method of claim 1 or claim 30, wherein the one or more nutrient media further comprise an auxin-related growth regulator.

33. The method of claim 30, wherein the antiethylene agent is a silver-containing compound, a silver complex or silver ion.

34. The method of claim 30, wherein the inhibitor of phenylpropanoid metabolism is selected from the group consisting of 3,4-methylenedioxynitrocinnamic acid, 3,4-methylenedioxycinnamic acid, 3,4-methylenedioxy-phenylpropionic acid, 3,4-methylenedioxyphenylacetic acid, 3,4-methylenedioxybenzoic acid, 3,4,-trans-dimethoxycinnamic acid, 4-hydroxycinnamic acid, phenylpropionic acid, fluorophenylalanine, 1-aminobenzotriazole, 2-hydroxy-4,6-dimethoxybenzoic acid, SKF-525A, ammonium oxalate, vinylimidazole, diethyldithiocarbamic acid, and sinapic acid.

35. (amended) The method of claim 1, claim 3, or claim 30, wherein the one or more nutrient media further comprises a polyamine.

36. The method of claim 35, wherein the polyamine is selected from the group consisting of spermine, spermidine, putrescine, cadaverine, and diaminopropane.

37. The method of claim 1 or claim 30, wherein the one or more nutrient media further comprise a taxane precursor.

38. The method of claim 32, wherein the auxin-related growth regulator is picloram, indoleacetic acid, 1-naphthaleneacetic acid, indolebutyric acid, 2,4-dichlorophenoxyacetic acid, 3,7-dichloro-8-quinolinecarboxylic acid, or 3,6-dichloro-o-anisic acid.

39. (amended) The method of claim 1, wherein the amount of said one or more taxanes recovered is at least 3-fold greater than the amount obtained from cells of *Taxus* species cultured without addition of any enhancement agents selected from the group consisting of (a) jasmonate-related compounds or alkyl esters thereof, (b) anti-ethylene agents, and (c) inhibitors of ^{phyl}propanoid metabolism.

40. (amended) The method of claim 1, wherein the amount of said one or more taxanes recovered is at least 5-fold greater than the amount obtained from cells of *Taxus* species cultured without addition of any enhancement agents selected from the group consisting of (a); ^{jas}monate-related compounds or alkyl esters thereof, (b) anti-ethylene agents, and (c) inhibitors of phylpropanoid metabolism.

41. (twice amended) The method of claim 1, wherein said one or more taxanes recovered is at least one compound selected from the group consisting of taxol, 7-epitaxol, 10-deacetyl-7-epitaxol, cephalomannine, 10-deacetyltaxol, 7-xylosyl-10-deacetyltaxol, baccatin III, and 10-deacetyl baccatin III.

42. (twice amended) The method of claim 1, wherein the cells are cultured in a first medium having a first composition, then the medium composition is changed to a second medium having a second composition which induces taxane production.

43. (amended) The method of claim 42, wherein the concentration of nitrate is lower in the second medium than in the first medium, and the concentration of saccharide is higher in the second medium than in the first medium.

44. (amended) The method of claim 43, wherein the first medium contains nitrate at a concentration which is 2 to 10 times the nitrate concentration in the second medium.

45. (amended) The method of claim 42, wherein the second medium contains saccharide at a concentration which is 2 to 5 times the saccharide concentration in the first medium.

46. (thrice amended) The method of claim 1, wherein the cells are cultured in media containing saccharide in a concentration of 1 – 150 g/L, nitrate ion in a concentration of 0.3 – 70 mM or a combination thereof.

47. (amended) The method of claim 43, wherein the first medium contains saccharide in the concentration of 1 – 30 g/L, and nitrate ion in the concentration of 2.5 – 70 mM; and the second medium contains saccharide in the concentration of 4 – 150 g/L, and nitrate ion in the concentration of 0.3 – 18 mM.

48. (amended) The method of claim 43, wherein the first medium contains saccharide in the concentration of 5 – 15 g/L, and nitrate ion in the concentration of 20 – 30 mM; and the second medium contains saccharide in the concentration of 35 – 55 g/L, and nitrate ion in the concentration of 2 – 7 mM.

49. (twice amended) The method of claim 42, wherein the medium which induces taxane production is replenished during cultivation by periodically replenishing nutrient medium components and removing spent medium.

50. The method of claim 1 or claim 30, wherein said step of cultivating further comprises exchanging nutrient medium at least once during the cultivation step.

51. The method of claim 1 or claim 30, wherein nutrient medium is the same for cell culture growth and for taxane production.

52. The method of claim 1 or claim 30, wherein cells of said *Taxus* species are cultivated by a continuous or semi-continuous process.

53. (amended) The method of claim 1, claim 3, or claim 30, wherein cells of said *Taxus* species are cultivated by a fed-batch process.

54. (twice amended) The method of claim 53, wherein the culture medium is replenished during cultivation by periodically replenishing nutrient medium components and removing spent medium.

55. (amended) The method of claim 1 or claim 30, further comprising the periodic removal of said at least one or more taxanes from the nutrient media.

56. The method of claim 1 or claim 30, wherein the *Taxus* species is selected from the group consisting of *T. canadensis*, *T. chinensis*, *T. cuspidata*, *T. baccata*, *T. globosa*, *T. floridana*, *T. wallichiana*, and *T. media*.

57. The method of claim 3 or claim 30, wherein the *Taxus* species is *Taxus brevifolia*.

58. The method of claim 1, wherein the cells are cultured in the presence of 0.03% to 15% v/v of carbon dioxide in the gas phase in equilibrium with the culture medium.

59. (amended) The method of claim 1 or claim 3, wherein the cells are cultured in the presence of 0.3% to 8% v/v of carbon dioxide in the gas phase in equilibrium with the culture medium.

60. The method of claim 1, wherein the cells are cultured in the presence of controlled oxygen concentration between 1% to 200% of air saturation.

61. The method of claim 1, wherein the cells are cultured in the presence of controlled oxygen concentration between 10% to 100% of air saturation.

62. (amended) The method of claim 1 or claim 3, wherein the cells are cultured in the presence of controlled oxygen concentration between 25% to 95% of air saturation.

63. The method of claim 42, wherein the second medium comprises a jasmonate-related compound or an alkyl ester thereof.

64. The method of claim 1 or claim 30, wherein a jasmonate-related compound or an alkyl ester thereof is added continuously to the cell culture.

65. The method of claim 1 or claim 30, wherein the one or more nutrient media contain glutamine.

66. (amended) The method of claim 3, wherein the cells are cultured in media containing saccharide in a concentration of 1 – 150 g/L, nitrate ion in a concentration of 0.3 – 70mM or a combination thereof.

67. The method of claim 1, wherein the one or more nutrient media contain an antiethylene agent.

68. A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein at least one of the one or more nutrient media comprises a compound selected from the group consisting of (a) amino acids and (b) polyamines.

69. (amended) The method of claim 68, wherein said amino acids, said polyamines, or a combination thereof are added to at least one of the one or more nutrient media.

70. A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein cells of said *Taxus* species are cultured in the presence of controlled oxygen concentration between 10% to 100% of air saturation.

71. The method of claim 2, wherein the concentration of silver ions, silver complexes, and silver-containing compounds is 0.01 μM – 10 μM .

72. A method for producing one or more taxanes in high yields in cell culture of a *Taxus* species comprising: cultivating in suspension culture, in one or more nutrient media under growth and product formation conditions, cells of a *Taxus* species derived from callus or suspension cultures, and recovering said one or more taxanes from said cells, said medium of said cell culture, or both, wherein β -phenylalanine is added to the one or more nutrient media.